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Quantitative determination of polysorbate 20 in nasal pharmaceutical preparations by high-performance liquid chromatography

Zsolt Őszi, Gábor Pethő *

Human Serum Production and Drug Manufacturing Co., Ltd., Godollo, H-2100 Tancsics M. 82, Hungary

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Abstract

A specific reverse-phase HPLC method has been developed for the quantitative determination of polysorbate 20 in various compositions of nasal solutions. This method is based on the acidic hydrolysis of the sorbitan laurate ester followed by the HPLC determination of the free lauric acid. Using this method, polysorbate 20 can effectively be separated and quantitatively determined in matrices containing a wide variety of preservatives, surfactants, and viscosity agents. Sample preparation involves a one-step hydrolysis with sulfuric acid and then a dilution with acetonitrile, prior to injection. The sample is analyzed on a 5-μm octadecylsilane reverse-phase column with a mobile phase of acetonitrile: 0.025 M aqueous di-Sodium hydrogen phosphate, pH = 2.8 (75:25). The column effluent is monitored by UV detection at 210 nm. The validity of the method has been verified with specificity, linearity, recovery, method- and system precisions data. The method is linear for polysorbate 20 from 2.5 to 125 mg ml^{-1} range. The limit of detection and limit of quantitation are 0.41 and 0.61 mg ml^{-1}, respectively.

Keywords: Polysorbate 20; Determination; Reverse-phase HPLC; Nasal solutions

1. Introduction

Polysorbates are widely used surfactants in pharmaceutical practice, cosmetics, skin care products and food. The polysorbates are non-ionic emulsifying agents formed by copolymerizing sorbitan anhydride and 20 moles of ethylene oxide. A fatty acid is esterified to one of the terminal hydroxyl groups of the polyoxiethylene-oxide side chains. The type of the attached fatty acid molecule is reflected by the numbers in the name of polysorbates. In case of the polysorbate 20 (Fig. 1) the fatty acid is lauric acid ($C_{11}H_{23}$–COOH).

The non-toxic polysorbate 20 [1] is used in metered dose nasal sprays to keep the drug dispersed in the propellant and to lubricate the actuator valve.

* Corresponding author. Fax: +36-28-420177; e-mail: pethog@human.gau.hu.

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There are several methods available to measure polysorbate 20. These include colorimetry [2], thin layer chromatography (TLC) [2], gas chromatography (GC) [2], high-performance liquid chromatography (HPLC) [3–5], and supercritical fluid chromatography (SFC) [6]. The TLC method is appropriately suitable for confirmation of the presence of polysorbates. The colorimetric method based on the complex formation with cobalt(II) thiocyonate ion is applicable for quantitative determination of polysorbates from various kinds of foods. However, colorimetric experiments with the nasal solutions did not allow to get the same reproducibility and recovery as indicated for either the standard polysorbates or food analyzed. The combination of TLC and complexation with cobalt(II) thiocyanate was further developed by McKean and co-workers [5] by using a normal-phase HPLC method; the solid phase was treated with cobaltothiocyanate solution. Such columns are not available commercially. The acid components of polysorbates can be measured by reverse-phase HPLC after a derivatization reaction with 2-nitrophenylhydrazone [3]. Volatile acid derivates can be analyzed by GC [2]. The supercritical-fluid chromatography method was successfully applied to analyze sorbitan trioleates. Direct analysis of the free acid component has not been published to date.

In this paper we report a reverse-phase HPLC method for the quantitative determination of polysorbate 20 among several commercially used surfactants, viscosity agents and additives present in metered-dose inhalers and nasal solutions. The method is based on the acidic hydrolysis of sorbitan laurate followed by a reverse-phase HPLC separation and UV detection of lauric acid. The hydrolytic reaction is complete within 24 h (Fig. 2). The sample preparation does not involve any transfer, instead, the addition of sulfuric acid to the sample followed by the addition of mobile phase takes place in the same flask or a test tube. The final acidic mixture can be injected directly on to the column. The presented method complies with the validation requirements of pharmaceutical industry.

2. Experimental section

2.1. Reagents and chemicals

The HPLC grade acetonitrile (ACN) was obtained from Fluka. Sulfuric acid, ortho-phosphoric acid, di-sodium hydrogen phosphate, citric acid (anhydrous), sodium citrate dihydrate, propylene glycol, benzalkonium chloride, polyethyleneglycol 400 from E. Merck, were used. Lauric acid and sorbitol were purchased from Sigma (St. Louis, MO). Benzethonium chloride and 2,6-Di-tert.-butyl-4-methylphenol (BHT) were obtained from Aldrich. The HPLC grade water was prepare from distilled water with a reverse osmosis, Millipore milli-Q System, (Millipore).

2.2. Instrumentation

The Waters chromatographic system was equipped with a 2690 separation module (Alliance) and a 486 tunable absorbance UV detector. The results were generated by Millennium 2020 chromatography manager (Waters). When checking instrument-to-instrument reproducibility, samples were measured with a system containing a SpectraSYSTEM P4000 pump, a SpectraSYSTEM AS3000 autosampler and a SpectraFOCUS forward optical scanning detector, results were generated by PC1000 software (thermo separation products). For the separation study the following five columns were applied: a 250×4 mm Purosphere RP-18 endcapped, 5 μm particle size (E. Merck), a 250×4 mm Lichrosphere 100 RP-8 endcapped, 5 μm particle size (E. Merck), a 250×4.6 mm Symmetry C18 (Waters),

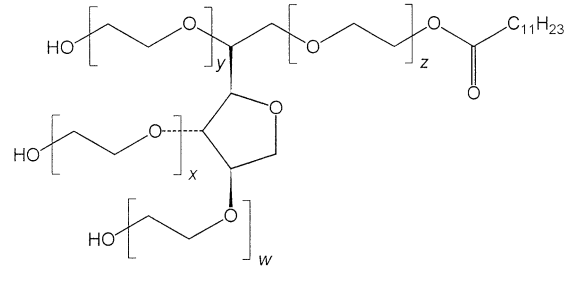

Fig. 1. The structure of polysorbate 20. $x + y + z + w = 20$.

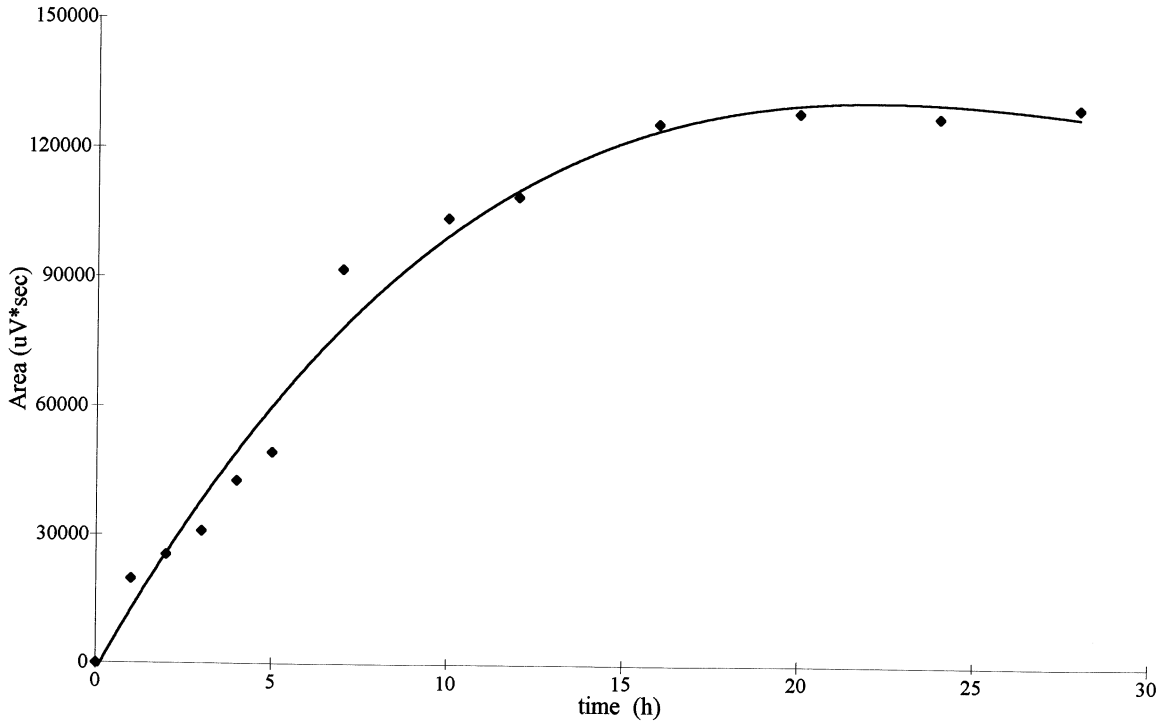

Fig. 2. Yield of hydrolysis (area count vs. time).

10 μm particle size, a 300 × 3.9 μBondapak C18 (Waters), 10 μm particle size, and a 250 × 4.6 zorbax RX-C18, 5 μm particle size (Hewlett-Packard). The UV spectra were recorded on a Perkin-Elmer Lambda20 UV/VIS spectrophotometer.

2.3. Mobile Phase

A 20 mM Na_2HPO_4 buffer solution was prepared with HPLC grade water and the pH was adjusted to 2.8 with diluted phosphoric acid. 250 ml buffer and 750 ml ACN was mixed, filtered through a 0.45 μm membrane filter, and sonicated to deareate for about 5 min.

2.4. Nasal solutions studied

During the study, five different nasal solutions (A, B, C, D, E) were prepared from a steroid type active ingredient (concentrations: A-0.25, B-0.10, C-0.15, D-0.10, E-0.10 mg ml^{-1}) and the following excipients with the indicated concentration ranges: polysorbate 20 (A-25, B-25, C-35, D-30, E-15 mg ml^{-1}), propylene glycol (50–75 mg ml^{-1}), polyethylene glycol 400 (100–200 mg ml^{-1}), benzalkonium chloride (0.3–0.35 mg ml^{-1}), butilated hydroxitoluene (0.1–0.125 mg ml^{-1}), disodium edetate (0.1 mg ml^{-1}), citric acid (0.05 mg ml^{-1}), sodium citrate · $2H_2O$ (0.0765 mg ml^{-1}), sorbitol (20 mg ml^{-1}). In one solution benzethonium chloride (0.1 mg ml^{-1}) was used instead of benzalkonium chloride. The pH was adjusted to 5.2.

2.5. Standard preparation

Accurately weighed, approximately 25 mg of polysorbate 20 was placed in a 10 ml stoppered glass tube and dissolved in 1 ml water. A total of 1 ml of 4 M Sulfuric acid was added. After 24 h reaction time at room temperature 4 ml ACN was added. A total of 20 μl standard solution was injected directly to the analytical column.

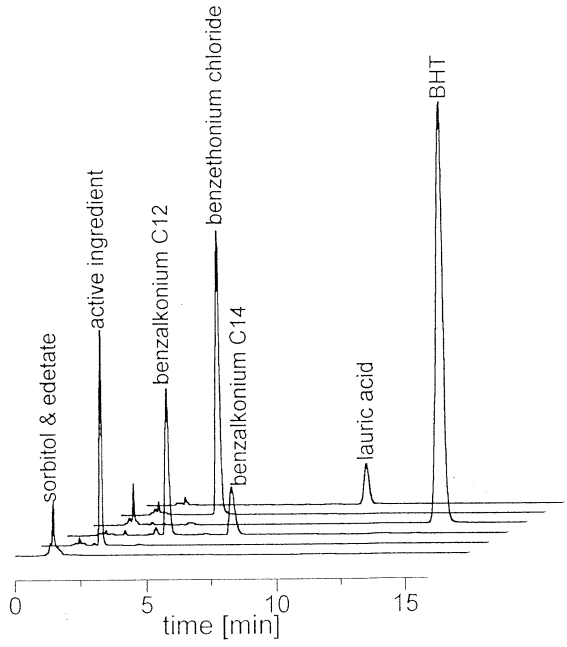

Fig. 3. Chromatograms of lauric acid (2 mg ml^{-1}), active ingredient (0.15 mg ml^{-1}), benzalkonium chloride (0,35 mg ml^{-1}), BHT (0.125 mg ml^{-1}), benzethonium chloride (0.1 mg ml^{-1}), sorbitol (20.0 mg ml^{-1}) and disodium edetate (0.1 mg ml^{-1}). Sorbitol and disodium edetate are eluted at t_0. Each chromatogram is from separate injections.

2.6. Sample preparation

A total of 1 ml nasal solution and 1 ml 4 M sulfuric acid were mixed, and treated the same way as described at standard preparation.

2.7. Chromatographic conditions

The mobile phase flow rate, 1.1 ml min^{-1}. All separation was performed at ambient temperatures. The column eluate was monitored at 210 nm, (flow cell 10 mm, 8 μl), a suitable wavelength obtained from UV spectrum of lauric acid.

2.8. Calculations

Integrated area counts were used to determine the amount of Polysorbate 20 in the assay preparation by external standard technique.

3. Results and discussion

A 12 point calibration curve showed linear detector response from 2.5 mg ml^{-1} to 125 mg ml^{-1} polysorbate 20 (slope: $2.46e+03$, intercept: $2.12e+02$, $R^2 = 0.999$,), thus, a single-point standard can be used with a label claim concentration of 25 mg ml^{-1}. Injection repeatability (system precision) was determined by six consecutive injections of a standard solution, with an RSD less than 2.0% in every case. The analysis repeatability (method precision) was measured by determining the polysorbate 20 content of a solution for three consecutive days, two separate samples prepared each day. The individual results obtained in mg ml^{-1} are as follows; day 1: sample #1: 25.478, sample #2: 26.056, day 2: sample #1: 25.365, sample #2: 25.395, day 3: sample #1: 25.628, sample #2: 26.013. The overall RSD has been found as 1.20%.

The method selectivity study was carried out by chromatographing the following solutions: 2 mg ml^{-1} lauric acid, 0.15 mg ml^{-1} active ingredient, preservatives in the following concentrations: benzalkonium chloride 0.35 mg ml^{-1}, BHT 0.125 mg ml^{-1}, benzethonium chloride 0.1 mg ml^{-1} and other additives: disodium edetate 0.1 mg ml^{-1}, sorbitol 20.0 mg ml^{-1}. Fig. 3 shows the appropri

Table 1
Statistical results of the recovery study

	80% of Label claim	100% of Label claim	120% of Label claim
1.	101.06	101.43	100.41
2.	102.45	98.41	97.97
3.	99.10	98.62	101.22
Mean:	100.87	99.49	99.90
RSD%:	1.67	1.69	1.62

Table 2
The chromatographic parameters using different columns

	Purosphere	Zorbax RX-18	Symmetry	Lichrosphere C8	μBondapak
Ret. time, min	8.15	8.26	11.29	6.14	7.38
Asym. Plate	9200	13900	12800	10700	6500
USP Tailing	1.09	1.10	1.35	1.10	1.43
Pressure, psi	1500	1100	1200	1070	550

Table 3
The recovery values, in %, for different columns

Solution	Purosphere	Zorbax RX-18	Symmetry	Lichrosphere C8	μBondapak
A	99.47	98.03	97.86	100.24	100.91
B	99.55	98.20	96.60	100.15	101.63
C	100.50	101.17	99.60	99.31	103.59
D	99.64	99.14	98.46	98.60	100.01
E	99.59	101.27	101.40	100.05	99.45

ate separation of the lauric acid from the other components.

Recoveries were determined by analyzing samples of 80, 100 and 120% of the label claim (approx. 20, 25, 30 mg ml^{-1} respectively). In each point three samples were prepared. In all samples the recovery was found within $\pm$ 3% of the theoretical value (Table 1).

Sample stability was proven by analyzing a sample (25 mg ml^{-1} polysorbate 20) for 25 h. No significant change in polysorbate 20 content was detected.

Limit of quantitation (LOQ) and limit of detection (LOD) were determined by diluting a Polysorbate 20 sample until the injection repeatability reached 5 and 10 RSD% respectively. The results gave LOQ = 0.61 mg ml^{-1} and LOD = 0.41 mg ml^{-1}.

Samples were analyzed under different chromatographic conditions. (For HPLC instruments and column specification, see instrumentation chapter.) The main parameters of the chromatographic separations by the different five columns are listed on Table 2. All five columns were suitable for polysorbate 20 analysis, the 5 μ, C18, columns in particular. Aliquots of the same samples were analyzed with all columns. The results in percent recovery of spiked amount of polysorbate 20 are shown in Table 3. All recovery data were acceptable.

Effects of the variation of mobile phase composition were studied by applying three different mobile phases containing 70, 75 and 80% ACN respectively; the peaks were well separated in every case. The appropriate instrument-to-instrument

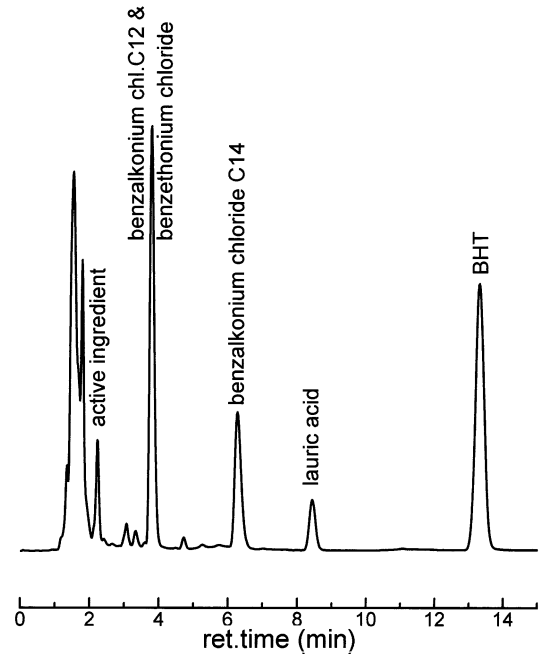

Fig. 4. A typical chromatogram of a nasal preparation containing 25 mg ml^{-1} polysorbate 20. For sample preparation, see text.

ruggedness of the procedure was studied by analysing the same samples on different instruments. No significant change in any results has been observed at this study. Analyst-to-analyst ruggedness was also checked with an outcome of no effect on the results. The final method (regarding linearity, selectivity, system precision, method precision and recovery studies) was developed with a chromatographic system containing an Alliance instrument, a Purospher column and a mobile phase described in experimental section. A series of nasal solutions (type A) was then measured by the final system. A fair RSD value of 2.1% was found from ten measurements. A typical chromatogram is shown in Fig. 4.

4. Conclusions

The simple method presented above has been found selective for the analysis of polysorbate 20. The analytical results in solution containing polysorbate 20 and different preservatives, surfactants and viscosity agents has been found appropriate for the chromatographic detection and quantitation of the targeted compound. The developed method was shown to be precise, selective and reproducible.

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